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Overview: Recent Developments in the Toxicity of Environmental Oxidants

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Recent results from several laboratories demonstrate convincingly that both man and animals respond to brief exposures of ozone at concentrations likely to be encountered in cities experiencing episodes of photooxidant air pollution. Acute exposures to nitrogen dioxide produce detectable changes at somewhat higher but not unrealistic concentrations. Oxygen at high concentrations is also toxic to laboratory animals, and rats can be made relatively resistant to 100% oxygen exposures by pre-exposing them to 85% oxygen for 5 days.

Enough time has elapsed since the initiation of these studies for a considerable body of data, both morphologic and biochemical, to have been generated. This information has been gathered in a number of different laboratories, each of which has studied the effects of different oxidants in different species for different times. One major purpose of this conference was to examine in detail the morphological and biochemical responses resulting from exposure to oxidants. Specific responses as a function of both species and toxicants may provide valuable clues in unraveling the mechanism of oxidant toxicity. Thus, the speakers were specifically charged to point out similarities and differences in results.

We must now carefully analyze the essential information and extract common factors. There are many more questions to be answered, but the framing and construction of these queries should await our analysis of the results to date. Our future questions should be oriented to the understanding of how the oxidant injuries effect and influence human disease, particularly lung disease. The observations discussed at this conference may contribute to an understanding of pathogenesis and by this to a logical means of prevention and therapy.

At the present time, short of literally "shutting down" a city, there are few if any steps that can be taken to ameliorate the responses to oxidant pollution. The California Air Pollution Emergency Plan promulgated by the state air resources board has published recommendations for self help during episodes of air pollution. These include avoiding strenuous outdoor activity, remaining indoors, not smoking, avoiding aerosols, dusts, etc. and avoiding traffic, etc.

Most of these steps are aimed at reducing the exposure rather than reducing the effect of exposure to a certain concentration. Several laboratories are now investigating the effects of xenobiotics on the biological responses resultant from exposure to photo-oxidants. Thus, a second purpose was to consider what is known about the effects of antioxidants and other compounds on oxidant toxicity and injury.

The first session considered morphologic lesions at the level of both the light and transmis-

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sion electron microscope with a number of scanning electron micrographs as well. The second session on biochemical effects was initiated by a review of the interaction of free radicals and biological systems. This paper was followed by others describing current results regarding biochemical indices of oxidant toxicity. With the exception of a paper describing the anticarcinogenic properties of vitamin A analogs, the last session was devoted to the effects of vitamin E on oxidant toxicity.

Exposure of mice to high oxygen concentrations for varying periods of time demonstrates that different pulmonary cell types are not equally susceptible to hyperoxia. With adequate exposure, the pulmonary vascular endothelium is injured and interstitial edema ensues, followed shortly thereafter by destruction and loss of type I alveolar epithelium. Alveolar macrophages, type II alveolar cells, and bronchiolar cells are less sensitive. DNA synthesis, as studied by thymidine labeling, is depressed during appropriate oxygen exposure but returns to normal after two days in air. The cell proliferation is predominantly in the endothelial and type II alveolar cells. No fibrosis is seen. The mechanism appears to be via direct access of oxygen diffusing across the alveolar membrane. One reason for the differential sensitivity may involve a loss of surface sialomucin in the susceptible cell membranes.

Unlike the oxygen lesions, continuous exposure of rats to 15 ppm NO_2 or 0.8 ppm O_3 for 3-5 months causes initial damage centered in the terminal bronchiole and proximal alveolar duct. The epithelium in this region is injured during the first 24 hr. The ciliated cells in the terminal bronchiole and the type I alveolar cells are most susceptible. Ciliated cells which do not die lose many ciliary processes. Even with continued exposure, regeneration occurs with nonciliated cells in the terminal bronchiole and type II alveolar cells in alveoli. These new cells are resistant to injury. Exposure to NO_2 produces changes in the terminal bronchioles including ciliated vacuoles, crystalloids and varied dense bodies which are not present with O_3. Ozone results in greater fibroblastic response, septal thickening, and an increased number of macrophages. The dog responds to chronic O_3 exposure with squamous metaplasia in the terminal airways and changes in the rough endoplasmic reticulum of the type II cells.

The effects of ozone on several animal species have been studied for periods of 7 to 90 days. Rats and monkeys are of about equal susceptibility to as low as 0.2 ppm ozone. These species also respond in the region of the terminal bronchiole and proximal alveolar duct or its equivalent. The changes include destruction of type I alveolar cells, replacement by type II alveolar cells, damage to both ciliated and nonciliated bronchiolar cells and accumulation of macrophages in the alveoli in this region. Mice, but not rats respond to 0.5 ppm ozone for 7 days with the development of nodular hyperplasia of Clara bronchiolar cells. Vitamin E deficiency potentiates these changes and the level of ozone at which short-term effects can be observed in the lungs is reduced. The phenomenon of adaptation has been studied by observing the effects of continued exposure to low-level ozone in rats. After 90 days of exposure to ozone for 8 hr daily, the lesions at any concentration studied are less severe than after 7 days of exposure (8 hr/day). This may be related to the persistance of altered type I cells which have some features of type II cells and may therefore be more resistant to oxidant lesion. Intermittent exposures with 6 day intervals for recovery show none of the adaptive changes or response.

The morphologic effects of ozone (0.2, 0.5, 0.8 ppm) and 80% oxygen have been compared for up to 7 days. Oxygen produced perivascular and interstitial edema, and endothelial changes, few macrophages, but little alterations in the Clara cell and ciliated cells of the terminal bronchiole. Wide differences in cell susceptibility are observed.

The effects of varying concentrations of oxygen, NO_2, and ozone on alveolar macrophage (AM) morphology have been investigated with the use of scanning electron microscopy. Exposure to 60% oxygen for 14 days or 100% oxygen for 48 hr disturbs the intricate surface membranes of the AM and produces membrane disruption, surface erosion, blebs, or fenestrae. NO_2 exposure produces no changes in morphology at 0.5 ppm for up to 24 weeks. Macrophages from mice exposed continuously to 2 ppm for 5 days/week show loss of surface process blebs and fenestrae and denudation or deterioration after 21 weeks of exposure. These changes are associated with significant decreases in phagocytic activity *in vitro* and increased susceptibility to infection *in vivo*.

Biochemistry of environmental oxidants has developed in two related directions; mechanism of injury and mechanism of defense against injury. The basic mechanism of injury from oxidants, including oxygen, appears to be related to

the highly reactive chemical state termed "free radicals." Many years ago, Gershman and Fenn recognized the similarity between injury due to radiation injury and that due to oxygen toxicity. Based upon this similarity they proposed that the mechanism of oxygen toxicity may involve free radicals. It now appears likely that the superoxide anion and other free radicals are produced by many metabolic reactions involving oxygen. Ozone and nitrogen dioxide also are likely to produce injury as free radicals.

The chemistry of free radicals is now being developed using isolated systems as well as some biological studies. The chemistry of free radicals can probably best be considered under three headings: (1) initiation of the process; (2) propagation; and (3) termination. The defense mechanisms in the lung may be directed to any of these three processes. For instance the availability of unsaturated fatty acids to the free radical may determine the quantity of lipid peroxides. The lung surface may have a relatively low fraction of esterified acids which are polyunsaturated and, therefore, the initiation of autoxidation by ozone or NO_2 in the lung may be less than for some models which are rich in unsaturated fatty acids. Enzymes such as superoxide dismutase may remove the free radicals before they damage the lung and the concentration of such enzymes may be of great importance. Antioxidants such as tocopherol may be factors in disrupting the process. Metabolic systems may be particularly important in protecting the lung from oxidant injury. For example the pentose pathway for glucose oxidation may have two important roles. This pathway is an important factor in producing NADPH from NADP in the cytosol of the cell. The NADPH may be important in reducing oxidants through the glutathione system or it may be important as a source of reducing equivalents necessary for synthesis of injured components of the cell. Therefore this pathway may play a role in reducing oxidants before injury occurs or it may be important in repair of injured cells.

The cells involved in changes which lead to the phenomenon termed adaptation are not definitely known but the type II cells in the alveolus are thought to be important. The number of these cells increases following injury from inhaled oxidants. It is somewhat surprising that the antioxidant, butylated hydroxytoluene (BHT) produces lung injury in mice which also leads to type II cell proliferation. This observation is consistent with the view that the type II cell proliferation is a response to any injury of the alveolar epithelium which probably damages the type I cells first and the proliferation of type II cells follows. After mice were given BHT the effect of exposure to 100% oxygen depended upon the stage of injury. Exposure to 100% oxygen reduces the proliferation during the repair phase. In this model it appears that changes of glucose-6-phosphate dehydrogenase correlate best with proliferation of the endothelial cells. However, other studies indicate that the activity of many enzymes changes after oxidant exposure, and we should not focus upon a single pathway.

These concepts of adaption to oxidant injury are useful when considering effects of environmental oxidants upon human lungs. Residents of Los Angeles, exposed to relatively high levels of atmospheric oxidants, appear to have adapted, in that acute exposures to inhaled oxidants in exposure chambers lead to less change of pulmonary function in residents of the Los Angeles Basin than in residents of other communities with less oxidants. It is not known whether such a change in sensitivity to inhaled oxidants (adaptation) implies no continuing lung injury.

The possibility of oxidant injury in the human subjects has been evaluated by observing blood changes which occur following oxidant inhalation. After acute oxidant exposure the erythrocyte has increased sensitivity to hemolytic stress, and some erythrocyte enzymes have increased activity. It appears that nonresidents of Los Angeles have greater changes of the erythrocyte after exposure to oxidants than do Los Angeles residents. The mechanism by which an inhaled highly reactive substance such as ozone can damage the blood is not understood, but it appears unlikely to be a direct transfer of ozone into the blood.

The similarity between the effects of irradiation and free radicals might also suggest that the increased frequency of cancer seen after exposure to irradiation may occur after exposure to free radicals. However, the concentration of free radicals in the cell nucleus and the defense mechanism against nuclear injury from free radicals have not been studied.

The studies discussed above establish the sequence of events in O_3 and NO_2 pathogenesis. In addition, other studies suggest that lipid antioxidants, particularly vitamin E may afford protection against O_3 toxicity. The anticarcinogenic properties of vitamin A and its analogs have been clearly demonstrated, the retinoids offering some degree of protection against chemical carcinogenesis in epithelia of bronchi, tranchea, stom-

ach, uterus, skin, and breast of experimental animals.

There is no doubt that decreased levels of vitamin E render animals more susceptible to oxidant toxicity. The question as to whether increased levels of vitamin E protect against oxidant toxicity has not yet been definitively answered. One must counter any suggestion of increasing the daily vitamin E intake with the fact that the effects of daily ingestion of two to three times the present daily requirement are unknown and could conceivably be more dangerous than exposure to oxidants.

The observations presented in this symposium indicate distinctive differences in the effects of the various oxidizing agents with regard to the sites of injury in the lung, the sequelae, the species and the time of exposures. This seems a most appropriate time to reevaluate our goals. We must refine our inquiries more specifically, limit the number of exposure models, if possible, evaluate the effects of long-term exposures and recoveries, and relate all of this to the problems of human lung disease. The studies summarized above and their abstracts which follow provide a clear picture of what is presently known about oxidant toxicity. Certain of these results suggest approaches to the prevention and therapy of lung damage induced by these ubiquitous air pollutants. Clearly further research is needed, both with animals and possibly with man. Hopefully, we are nearing the stage where we can consider evaluation of some of these preventative agents in test animals and in human populations at risk during exposure to photooxidants.